Short Notes

Capsella bursa-pastoris seeds. Are they "carnivorous"?

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A recent article by Joe Mazrimas (1977) asked the question "Did you ever hear of carnivorous seeds?" The answer was probably an emphatic "No"! While the idea may have some intrinsic appeal, it is almost paradoxical in that one is so accustomed to thinking of carnivory in terms of adult plants and mechanisms which usually involve something more obvious and active than a seed, which just sits there. Nevertheless, it is the purpose of this article to suggest that certain plant seeds, particularly those of Shepherd's Purse (*Capsella bursa-pastoris*), have all of the necessary capabilities for carnivory.

Initially our work had been aimed at determining the potential of mucilaginous seeds (i.e. those which release a gummy covering or pellicle upon immersion in water – see Hyde, 1970 for a description of the anatomy and mechanism of mucilage release) for the biological control of mosquito larvae. The original observation of Reeves and Garcia (1969) that larvae became attached to such seeds and subsequently died was confirmed by Barber *et al.* (1974). The phenomenon is impressive when one can observe up to 20 larvae attached to a single seed which is little larger than a pinhead. The accompanying photo shows that larvae can become attached to the extent that the seed itself becomes completely obscured. Observations such as these were sufficiently dramatic as to prompt further investigation. It rapidly became evident that the interaction between seeds and larvae was more complex than at first appeared.

First, a survey of various seeds which have mucilaginous pellicles (principally members of the Cruciferae-Mustard family)revealed that they were not equally capable of entrapping larvae. Chemical analyses of the mucilages from different species of seeds indicated that a cellulose moiety was necessary for the mucilage to be "sticky", insofar as mosquito larvae was concerned. Those seeds whose mucilage lacked this cellulose fraction were unable to entrap larvae (Barber *et al.*, 1974).

Second, it has been demonstrated unequivocably that certain species of mucilaginous seeds upon immersion in water, release an attractant which promotes positive chemotaxis in mosquito larvae (Barber and Page, 1975). This chemoattraction was immediate and strongest in seeds which possessed a "sticky" pellicle. Seeds with a "non-sticky" pellicle evoked positive chemotaxis only after relatively long periods of immersion in water. Non-mucilaginous seeds generally showed no evidence of chemoattraction or only after prolonged soaking (Page and Barber, 1975).

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Larvae of the mosquito *Culex pipiens quinquefasciatus* attached to a seed of *Capsella bursa-pastoris.* Each larva is attached by its oral brushes to the mucilaginous pellicle surrounding the seed which is obscured by the heads of the larvae. Photograph by Dr. L. Y. Yatsu, Southern Regional Research Lab.. U. S. D. A.. New Orleans. La.

Third, it became apparent that larvae which had become entrapped by the mucilaginous seeds died at a much faster rate than they "had any right to". While a minute seed attached to the oral brushes of a larva may inhibit feeding, it does not appear to cause stress in terms of exhaustion, 0_2 deprivation, etc. The inference then was that a toxin was being released by the seeds; this was duly demonstrated by preventing attachment of larvae to seeds while maintaining aqueous contact between the two. Under these circumstances, the larvae died significantly faster than did larvae in the complete absence of seeds (Page and Barber, 1974).

At this point, some "strategy" on the part of the seeds seemed to be emerging. The seeds had a means of attracting, entrapping and killing prey but unless they had some use for the prey then there appeared to be little reason for the preliminaries. Therefore proteolytic activity was looked for using the method of Nelson *et al.* (1961). It was found that protease(s) were indeed liberated upon imbibition of *C. bursa-pastoris* seeds and that the proteolytic activity was confined to the mucilage (i.e. the site of prey attachment). Further, it was shown that germinating seeds were able to take up and incorporate labelled amino acids, indicating, presumably, that had these seeds been provided with a protein source (prey) they would, using their own protease(s), have been able to hydrolyze these proteins, making amino acids available for uptake and growth.

Thus, seeds of *C. bursa-pastoris* appear to have all of the necessary prerequisites for carnivory, at least as far as mosquito larvae are concerned (Barber and Page, 1976). They are able to attract, entrap, kill and digest prey. Further, they are able to take up the products of the digestion and utilize them to nourish the growing seedling. However, since *C. bursa-pastoris* seeds would seldom, if ever, encounter a mosquito larva under natural circumstances, then these phenomena can have little biological significance unless they are also effective against more "normal" prey i.e. organisms that the seeds could be expected to encounter under natural conditions. This possibility is currently being tested using such organisms as motile soil bacteria, nematodes and protozoans. While the results to date are still incomplete it is becoming clear that *C. bursa-pastoris* seeds are able to at least attract and kill certain of these organisms; entrapment appears to be less likely. However, if the seeds can attract and kill prey then actual entrapment would be somewhat superfluous anyway.

With regard to soil nematodes, samples of seeds were buried and at various time intervals thereafter (up to 7 days) they were recovered (as many as could be found) and examined under the microscope for associated nematodes. The numbers of nematodes associated with the seed samples were compared with the number of nematodes associated with an identical number of similarly-sized soil and organic matter samples. The results showed a very clear preference of the nematodes for the seeds with ratios of up to 15:1 (numbers of nematodes per seed sample/numbers of nematodes per soil sample) being common. These results have been substantiated by lab experiments under more closely controlled conditions. The apparatus used was a modified, scaled-down, version of that used to demonstrate the attraction of *C. bursa-pastoris* seeds for mosquito larvae (Page and Barber, 1975). The distributions of nematodes (pure cultures of *Rhabditis* sp. and mixed natural populations obtained from soil) in a small (20 x 4 x 3 mm deep) plexiglass trough were determined at hourly intervals, up to 24 hours in the presence and absence of *C. bursa-pastoris* seeds. When no seeds were present in the trough the nematodes distributed themselves randomly throughout. In the presence of seeds, the nematodes accumulated in statistically significant numbers in the area of the trough which contained the seeds.

The effect of the seeds upon nematode survival was determined by placing identical populations of nematodes in depression slides. Seeds (2) were added to certain depressions and not to others. The populations were monitored over a period of eight days at which time only 25% of the nematodes in the presence of seeds remained alive as compared with 93% of those in the absence of seeds.

Similar results have been obtained using cultures of the protozoan *Colpidium striatum*. An H-shaped tubular apparatus was devised in which samples of the protozoan culture could be introduced into the center of the horizontal cross arm. The protozoans were then free to swim in either direction which they did in equal numbers to each vertical arm. However, when *C. bursa-pastoris* seeds were introduced into one of the vertical arms, say the right, then more than three times as many protozoans swam to the right than swam to the left. These methods have also been used to demonstrate the positive chemotaxis of the motile soil bacterium *Escherichia coli* to seeds of *C. bursa-pastoris*.

Evidence for the enhanced mortality of protozoans and bacteria in the presence of *C. bursa-pastoris* seeds is still preliminary but indicates that a toxin for these organisms is released by the seeds, upon imbibition.

The evidence therefore is strong that *C. bursa-pastoris* seeds are able to attract nematodes, protozoans and bacteria. It is also strong that they are able to cause increased mortality in nematodes; this also appears to be true for protozoans and bacteria, but the evidence is less complete here. Therefore, it appears that the sequence of events that has been well demonstrated using mosquito larvae is also possible for organisms that form a natural part of the seed's environment. It can be assumed that, having attracted and killed the prey, whether that is a mosquito larva, a nematode, a protozoan, or whatever, the seed's protease(s) is just as effective in digesting the prey's protein as it was in digesting the protein provided in the Nelson *et al.* (1961) assay. Similarly, one can assume that the amino acids so liberated can be taken up, incorporated and utilized for growth just as well as were the labelled free amino acids which came from a bottle.

The question now arises, does this all add up to carnivory? Certainly the potential seems to be there and one can fairly easily envisage circumstances under which germinating *C. bursa-pastoris* seeds would be able to supplement their nutritient with organic nitrogen derived from attracted, entrapped, killed and digested prey. But why would they need to do so when a seed is usually thought of as being a self-sufficient entity? Many seeds having mucilaginous pellicles are found in nutritionally poor environments (Young and Evans, 1973) though *C. bursa-pastoris* itself is a relatively ubiquitous weed. In addition, the small size of the seeds (approximately 2 million/ lb. in the case of *C. bursa-pastoris*) makes them incapable of storing large amounts of endogenous food. Therefore, any plants that have acquired the ability to attract exogenous nutrients, as early as in the seed stage, would have a decided selective or competitive advantage. However, is all of this sufficient justification to apply the term carnivory? I wonder if proving carnivory is not a little like proving a crime, i.e. one must show motive, method and opportunity (eyewitnesses are helpful but not essential). Much of this has been demonstrated (or may be logically assumed) for *C. bursa-pastoris* seeds. Nevertheless, I am still somewhat reluctant to use the term carnivory and when it has been necessary I have tried to cover myself by using quotes — as in this article's title. I am encouraged to note, however, that even those who have worked with carnivorous plants longer and are more familiar with them than I, can question whether a particular plant is or is not truly carnivorous, e.g. Rose (1977) discusses "Is *Byblis* carnivorous?", similarly the article by Olivet and Mirimanoff (1940) is entitled "*Pinguicula vulgaris* L., est elle une plant carnivore?" For this reason I was pleased to see the article "Are carnivorous plants carnivorous?" by Williams (1975). *C. bursa-pastoris* seeds have been shown to fulfill all but one of the criteria for carnivory listed by Williams (1975); that one is that fed plants "prosper more than unfed control plants". This is a difficult determination to make since it does not involve life or death but rather the qualitative judgment of whether a fed plant is healthier in some respect than is an unfed plant. *C. bursa-pastoris* seeds do not appear to *need* prey in order to germinate and for the seedlings and subsequent plants to be quite healthy. However, neither do such accepted carnivorous plants as *Pinguicula* and *Drosera* (Harder and Zemlin, 1967; Harder, 1964). The concluding paragraphs of Williams (1975) indicate that a certain amount of semantics is involved in "carnivory". Given this and the various properties that *C. bursa-pastoris* seeds have been shown to possess, I leave it to the readers of CPN to judge whether or not they (the seeds, not the readers) are "carnivorous".

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